LINKAGE OF THE FACTOR FOR BIFID WING. THE BIFID WING AND OTHER SEX-LINKED FACTORS IN DROSOPHILA.

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The experiments described in the following pages were made primarily in order to test whether crossing-over of factors has any subsequent effect on the linkage relations of the factors involved. For instance, if a red eyed fly with bifid wings is crossed to a white eyed fly with normal wings there will appear in F_2 the non-crossover classes, red bifid and white normal, and also some crossovers white bifid and red normal. These cross-overs (white bifid) were then used to determine whether the same linkage values would reappear in their grandchildren; in other words, whether a crossover in a particular place predisposes to more frequent crossing-over in the same place.

I wish to take this opportunity of acknowledging my indebtedness to Professor Morgan and to Mr. Bridges for their advice and suggestions.

The term "bifid" was given to a type of wing in which the second inner vein fails to reach the margin, often producing a bifid or forked wing.[1] The forked condition is variable. A constant feature, however, which the forked condition frequently accompanies, is a fusing of the wing veins at the base of the wing. Flies possessing this characteristic cannot fly.

The following abbreviations are used in the tables to denote the factors taken account of in the flies studied:

For eye color—R = red, V = vermilion, W = white;

For body color—Gr = Gray, Y = yellow;

For wing shape—L = long, Bf = bifid;

X = factor to which sex linked factors are linked;

o = gamete not possessing X-factor.

The factors R, V, Gr and Bf are in association with the X-factor which is duplex in the female and simplex in the male.

[1] Morgan, *Science*, Vol. 35, March 12, 1912.

For the sake of simplicity these factors will be considered in this paper as single units.

Experiment I.

A long-winged vermilion eyed female was mated with a bifid winged red eyed male. The results to be expected in the F^1 and F^2 generations are shown in Table I.

Table I.

To Illustrate Experiment I.

P^1	LVX – LVX = LV ♀	BfRX – o = BfR ♂
	P^1 Eggs LVX	Sperm BfRX, o
F^1	LVX – BfRX = LR ♀	LVX – o = LV ♂
	F_1 Eggs { (1) LVX, (2) BfRX, (3) LRX, (4) BfVX,	Sperm { LVX, o
F^2	LVX – LVX, BfVX – LVX } = LV ♀	LVX – o = LV ♂
		BfRX – o = BfR ♂
	BfRX – LVX, LRX – LVX } = LR ♀	LRX – o = LR ♂
		BfVX – o = BfV ♂

The F^1 generation consisted of LR females and LV males. The actual results of the F^2 generation are shown in Table II.

Table II.

Results of Experiment I.

F^2	♀		♂				Total ♂ ♂	Per Cent. of Crossovers.
	LR	LV	BfR	LV	BfV	LR		
	273	256	123	144	50	62	379	30

All the combinations expected are realized. Owing, however, to the coupling in pairs of the P^1 factors, the numbers of males in the two classes consisting of the combinations LR and BfV are fewer than those of the other two classes. That they appear at all is evidence of the incompleteness of the linkage, the new combinations being due to a rearrangement or crossing over of factors within the germ cells of the F^1 flies. The strength of linkage may be determined by that percentage of the total which are crossovers, in this case 112/379 or approximately 30 per cent.

On mating the F^2 males possessing the new combination BfV

with normal red eyed long winged flies (LR) we obtain LR male and female offspring. When these are interbred we obtain the F^2 results shown in Table III.

Table III.

Results of Experiment I., Crossover.

F^2	♀	♂				Total ♂♂	Per Cent. of Crossovers.
	LR	BfV	LR	LV	BfR		
	498	80	123	71	53	327	38

These results even with comparatively small numbers show that the new combination BfV produced by a crossing over of factors possesses approximately the same strength of coupling as did the original.

Experiment II.

In the reciprocal cross, BfR females with LV males, the offspring consist of LR females and BfR males. In the second generation the four classes of males occur in the same proportionate numbers as those in Experiment I.

The results to be expected are shown in Table IV.

Table IV.

To Illustrate Experiment II.

P^1	BfRX – BfRX = BfR ♀	LVX – o = LV♂
	P^1 Eggs BfRX	Sperm LVX, o
F^1	BfRX – LVX = LR ♀	BfRX – o = BfR♂
	F_1 Eggs $\left\{\begin{array}{l}\text{(1) BfRX, (2) LVX,}\\ \text{(3) BfVX, (4) LRX,}\end{array}\right.$	Sperm $\left\{\begin{array}{l}\text{BfRX, o}\\ \end{array}\right.$
F^2	$\left.\begin{array}{l}\text{BfRX – BfRX}\\ \text{BfVX – BfRX}\end{array}\right\}$ = BfR♂	BfRX – o = BfR♂
		LVX – o = LV♂
	$\left.\begin{array}{l}\text{LVX – BfRX}\\ \text{LRX – BfRX}\end{array}\right\}$ = LR ♀	BfVX – o = BfV♂
		LRX – o = LR♂

The actual results of the experiment are shown in Table V.

Table V.

Results of Experiment II.

F^2	♀		♂				Total ♂♂.	Per Cent. of Crossovers.
	LR	BfR	LV	BfR	LR	BfV		
	103	108	57	85	31	33	206	31

Experiment III.

Experiment III. confirms the results of Experiment I. LV females were crossed with BfR males. The F[1] females, all of which possess the gametic constitution, LVX–BfRX, (see Table I.), instead of being allowed to breed with their F[1] brothers, were mated with normal wild males possessing the gametic constitution RLX–o. The results are shown in Table VI. Although the cultures show a rather wide range of fluctuation it is doubtful if any of these are significantly different from 32, which is the value given by the total.

Table VI.

Results of Experiment III.

LV♀ × BfR♂							
F[1]		LR♀♀ and LV♂♂					
		LR♀ of F[1] × pure LR♂					
F[2]		♀	♂				Per Cent. of Crossovers.
Bottle No.	Transfer.	LR	LV	BfR	LR	BfV	
1	a	185	70	44	18	13	
	b	120	37	33	18	15	
	c	72	12	24	8	12	
		377	119	101	44	40	28
2	a	133	40	39	20	16	
	b	93	34	26	18	11	
		226	74	64	38	27	32
3	a	150	50	50	22	18	
	b	97	33	31	14	13	
		247	83	81	36	31	29
4	a	156	50	39	19	17	
	b	105	32	30	22	13	
		261	82	69	41	30	32
5	a	118	26	26	19	13	
	b	94	21	21	15	16	
	—	212	47	47	34	29	40
6	—	155	71	27	16	15	24
7	—	181	58	42	21	22	30
8	—	199	51	58	30	28	35
9	—	112	21	16	15	9	39
Totals		1970	606	485	275	231	32

Experiment IV.

LY females were mated with BfGr males. The F[1] generation consisted of LGr females and LY males. Interbreeding these

gave rise to the four possible classes of males as shown in Table VII.

Table VII.

To Illustrate Experiment IV.

P^1	LYX – LYX = LY ♀	BfGrX – o = BfGr ♂
	Gametes LYX	BfGrX, o
F^1	LYX – BfGrX = LGr ♀	LYX – o = LY ♂
	F_1 Eggs { (1) LYX, (2) BfGrX, (3) LGrX, (4) BfYX	Sperm { LYX, o
F^2	LYX – LYX, BfYX – LYX } = LY ♀	LYX – o = LY ♂
		BfGrX – o = BfGr ♂
	LGrX – LYX, BrGrX – LYX } = LGr ♀	LGrX – o = LGr ♂
		BfYX – o = BfY ♂

The actual results of the experiment are shown in Table VIII.

Table VIII.

Results of Experiment IV.

P^1 LY ♀ × BfGr ♂

F^1 LGr ♀ × LY ♂

F^2	♀		♂				Per Cent. of Crossovers.
Bottle No.	LGr	LY	BfGr	LY	BfY	LGr	
1	69	29	46	41	0	7	
2	53	43	34	23	1	1	
3	70	59	57	46	5	1	
4	93	71	77	76	2	4	
Totals	285	202	214	186	8	13	5

Table VIII. shows that the linkage of the factors for bifid and yellow is stronger than for bifid and vermilion studied in Experiment I., the per cent. of crossovers being 5.

On mating males of the crossover class BfY with pure LR females we find in the F^2 generation (Table IX.) that the new combination persists in the same percentage as did the original combinations depicted in Table VIII. These results are in harmony with those obtained in Experiment I.

Table IX.

Results of Experiment IV. The Reciprocal Cross.

P^1	BfGr ♀ × LY ♂					
F^1	LR ♀ × LR ♂					
F^2	♀	♂				Per Cent. of Crossovers.
Bottle No.	LGr	LGr	BfY	LY	BfGr	
5	126	67	53	3	5	
6	237	110	99	7	3	
7	102	50	37	2	1	
8	174	60	59	2	2	
Total	649	287	248	14	11	4.5

Experiment V.

This experiment is a repetition, during the following summer, of Experiment IV. The results of both reciprocal crosses are given in Tables X. and XI.

In order to secure large numbers the parent flies were transferred to fresh bottles every ten days. The transfers are indicated in the second column.

Table X.

Results of Experiment V. Repetition of Experiment IV. (Cf. Table VIII.)

F^2		♀		♂				Per Cent. of Cross-overs.
Bottle No.	Transfer.	LGr	LY	BfGr	LY	BfY	LGr	
1	a	27	26	35	20	4	3	
	b	96	84	82	81	1	1	
		123	110	117	101	5	4	
2	a	54	51	48	42	4	1	
	b	45	31	33	26	4	3	
	c	61	49	45	36	1	4	
		160	131	126	104	9	8	
3	a	113	113	93	79	10	4	
	b	167	150	149	114	8	7	
		280	263	242	193	18	11	
4	a	19	32	18	16	1	1	
	b	78	66	75	73	7	4	
	c	232	69	198	64	6	9	
	d	97	12	77	11	—	7	
		426	179	368	164	14	21	
Totals		989	683	853	562	46	44	6

The results of the reciprocal cross are shown in Table XI.

Table XI.

Results of Experiment V. The Reciprocal Cross. (Cf. Table IX.)

F^2		♀		♂				Per Cent. of Cross-overs.
Bottle No.	Trans-fer.	LGr	BfGr	LY	BfGr	LGr	BfY	
5	a	33	38	24	30	2	3	
	b	88	51	77	73	6	5	
	c	59	40	36	33	1	0	
	d	55	37	43	46	5	5	
		235	166	180	182	14	13	
6		194	146	111	148	16	5	
7		75	39	50	53	0	4	
8		121	114	78	82	4	4	
9		104	96	40	69	4	4	
Total		729	561	459	534	38	30	6

The percentage of crossovers is the same as that shown in Table X. and slightly larger than that in the corresponding experiment in Table IX.

Experiment VI.

LW females were mated with BfR males.

The LR females and LW males of the F^1 generation were allowed to interbreed. Table XII gives the numbers produced in the resulting F^2 generation. The percentage of crossovers is approximately 8.

Table XII.

Results of Experiment VI.

	LW♀ × BfR♂						
F^1	LR♀ × LW♂						
F^2	♀		♂				Per Cent. of Crossovers.
Bottle No.	LR	LW	LW	BfR	LR	BfW	
1	218	182	194	181	16	12	7
2	94	85	100	103	15	15	13
3	115	86	69	77	2	5	5
Total	427	353	363	361	33	32	8

The strength of coupling of the new combinations in the cross-overs was determined by mating the crossover BfW males with pure LR♂. Table XIII. gives the results of this mating.

TABLE XIII.

RESULTS OF EXPERIMENT VI. CROSSOVERS.

LR♀ × BfW♂						
F^2	LR♀ × LR♂					
F^2	♀	♂				Per Cent. of Crossovers.
Bottle No.	LR	LR	BfW	LW	BfR	
1	329	144	111	11	12	7
2	394	166	145	15	15	9
3	193	96	64	5	7	7
4	438	173	147	21	18	11
5	152	74	51	7	8	11
6	301	106	58	6	6	7
Total	1807	759	576	65	66	9

The crossing back of the crossovers occurs in approximately the same percentage as did the crossovers to the original combinations in Experiment VII.

The results here harmonize with those of Experiment I. We may legitimately infer that the large classes in the F^2 generation are those with the combinations occurring in the grandparents no matter whether the grandparents have acquired those factors early or late in their phylogenesis.

EXPERIMENT VII.

The results of the reciprocal cross, BfR females by LW males is shown in Table XIV.

TABLE XIV.

RESULTS OF EXPERIMENT VII. RECIPROCAL OF EXPERIMENT VI.

BfR♀ × LW♂								
F^1	LR♀ × BfR♂							
F^2		♀		♂				Per Cent. of Cross-overs.
Bottle No.	Trans-fer.	LR	BfR	LW	BfR	LR	BfW	
1	a	161	99	108	105	6	7	
	b	135	77	100	87	4	6	
	c	18	10	17	14	0	1	
		314	186	225	206	13	14	6
2		76	54	85	65	3	4	4
3		117	91	86	83	6	4	6
4		166	136	130	146	5	3	3
5		157	119	113	129	3	2	2
6		174	142	113	120	5	3	3
7		111	80	81	83	2	3	3
8		120	75	64	94	3	7	6
9		143	117	107	107	5	4	4
Total		1378	1000	1004	1033	45	44	4

The number of crossovers is far too small to give the same ratio found in Experiment VI. Here the percentage is only 4.

Experiment VIII.

This is a modification of Experiment VI. LR females were mated with BfW males. The F^1 generation consisted of LR males and females. The LR ♀ were removed from their brothers and crossed with pure BfW males. This was done in order to secure four possible classes, in the F^2 generation, not only of males but also of females thus rendering the female counts also available for study.

Table XV. shows the gametic constitution of the flies used and the combinations expected in the F^1 and F^2 generations.

Table XV.

To Illustrate Experiment VIII.

P^1	LRX – LRX = LR ♀	BfWX – o = BfR ♂
	Gametes LRX	BfWX, o
F^1	LRX – BfWX = LR ♀	LRX – o = LR ♂
	LR ♀ of F^1 × BfW ♂ (from stock)	
	F_1 Eggs { (1) LRX, (2) BfWX, (3) LWX, (4) BfRX	Sperm { BfWX. o
F^2	LRX – BfWX = LR ♀	LRX – o = LR ♂
	BfWX – BfWX = BfW ♀	BfWX – o = BfW ♂
	LWX – BfWX = LW ♀	LWX – o = LW ♂
	BfRX – BfWX = BfR ♀	BfRX – o = BfR ♂

In Table XVI. are given the actual results of the experiment. Judging from the total numbers of the F^2 flies the break in the coupling occurs in the ratio of 5 per cent. in both females and males. Only the normal range of variations from this are apparent when the numbers from individual bottles are considered.[1]

[1] In the previous experiments 4–6 females were placed with as many males in the same bottle. For Experiments VIII. and IX. one female with 2–3 males was placed in a bottle. She and the males were transferred every 6–8 days. Some of the females lasted for six weeks by which time they were fairly exhausted of eggs.

Table XVI.

Results of Experiment VIII.

LR ♀ × BfW ♂

F^1 LR ♂

LR ♀ of F^1 × BfW ♂ (from stock bottle).

F^2 Bottle No.	F^2 Transfer	♀ LR	♀ BfW	♀ LW	♀ BfR	♀ Per Cent. of Crossovers.	♂ LR	♂ BfW	♂ LW	♂ BfR	♂ Per Cent. of Crossovers.
1	a	45	30	3	2		36	35	3	1	
	b	39	26	4	1		56	26	1	4	
	c	83	83	5	7		66	92	3	5	
	d	63	53	1	6		61	76	7	4	
	e	15	12	0	0		17	13	2	1	
	f	2	1	0	0		1	0	0	0	
		247	205	13	16	6	237	242	16	15	6
2	a	40	19	0	2		35	34	0	2	
	b	45	55	3	1		50	44	3	3	
	c	66	75	4	1		67	75	2	3	
	d	51	46	5	4		48	39	3	2	
	e	33	39	2	1		32	34	4	2	
		235	234	14	9	5	232	226	12	12	5
3	a	52	35	4	1		29	47	4	1	
	b	20	24	3	0		29	23	1	1	
	c	34	28	4	1		44	25	0	3	
	d	29	22	2	1		23	29	0	1	
	e	10	11	3	0		8	12	2	1	
		145	120	16	3	7	133	136	7	7	5
4	a	88	90	6	3		94	102	10	1	
	b	105	101	8	4		90	122	4	5	
	c	54	38	0	2		59	49	3	3	
	d	28	37	7	3		32	33	2	3	
	e	23	12	1	1		17	14	2	1	
		298	278	22	13	6	292	320	21	13	5
5	a	57	70	4	1		59	71	1	3	
	b	38	33	4	1		38	41	0	2	
	c	24	33	1	0		23	24	0	2	
	d	19	18	1	0		14	14	1	2	
		138	154	10	2	4	134	150	2	9	4
6		103	73	7	6	7	97	79	6	4	5
7		38	30	0	2	3	56	45	2	3	5
8		120	93	8	7	7	90	125	6	4	4
9		209	190	5	5	2	185	169	13	14	7
10		88	66	5	1	4	61	79	7	3	7
11		63	60	5	3	6	95	77	5	7	7
12		162	143	8	5	4	149	129	9	11	7
13		162	141	8	7	5	170	159	7	8	4
14		156	138	10	6	5	196	157	12	7	5
15		139	116	5	4	3	97	111	6	4	5
16		181	155	13	5	7	171	154	8	9	5
Total		2322	2053	141	89	5	2246	2229	130	119	5

EXPERIMENT IX.

For this experiment both males and females with crossed over factors were used. These were taken from the F^2 generation produced in Experiment VIII. On looking over Table XV. it will be noticed that the F^2 BfR females possess the gametic constitution of BfRX–BfWX. Both of the combinations BfR and BfW have been produced in the normal percentage by a crossing over in the F^1 generation of factors originally coupled in the grandparents.

The F^2 LW males have been similarly produced, their factors L and W being crossovers.

All combinations, therefore, introduced into the F^1 generation have been produced by a rearrangement of the original combinations owing to crossing over.

Table XVII. shows the expected results of such a crossing.

TABLE XVII.

TO ILLUSTRATE EXPERIMENT X. (CROSSOVERS.)

P^1	BfRX – BfWX = BfR ♀	LWX – o = LW ♂
	Gametes BfRX, BfWX	LWX, o
F^1	BfRX – LWX = LR ♀	BfRX – o = BfR ♂
	BfWX – LWX = LW ♀	BfWX – o = BfW ♂
	LR of F^1 × BfW of F^1	
	F_1 Eggs { (1) BfRX, (2) LWX, (3) BfWX, (4) LRX	Sperm { BfWX, o
F^2	BfRX – BfWX = BfR ♀	BfRX – o = BfR ♂
	LWX – BfWX = LW ♀	LWX – o = LW ♂
	BfWX – BfWX = BfW ♀	BfWX – o = BfW ♂
	LRX – BfWX = LR ♀	LRX – o = LR ♂

In spite of the conditions of the experiment the combinations introduced into the F^1 generation persist with a strength of linkage approximately equal to that existing among the original combinations.

Table XVIII. gives the actual results of the experiment.

Table XVIII.

Results of Experiment X.

BfR ♀ × LW♂ (Both crossovers from F² of Table 16).

F¹ LR and LW♀♀, BfR and BfW♂♂

LR♀ of F¹ × BfW♂ (from stock bottle)

F²		♀					♂				
Bottle No.	Transfer	BfR	LW	BfW	LR		BfR	LW	BfW	LR	
17	a	89	62	1	3		78	64	5	2	
	b	46	39	1	0		41	46	0	1	
	c	33	31	0	2		26	32	3	3	
	d	39	46	2	3		33	32	2	1	
	e	28	28	0	5		23	29	4	3	
	f	2	0	0	0		2	4	0	0	
		237	206	3	13	3	203	207	14	10	6
18	a	32	37	3	3		38	30	3	0	
	b	56	41	3	2		56	48	5	2	
	c	21	22	3	0		21	15	2	0	
	d	31	29	2	3		32	24	5	4	
	e	27	31	0	3		38	28	3	2	
	f	6	5	1	0		4	5	0	0	
		173	165	12	11	6	189	150	18	8	7
19	a	49	57	2	5		67	62	3	1	
	b	30	30	2	3		26	29	2	2	
	c	28	23	2	3		22	18	3	1	
	d	37	43	0	4		38	32	5	1	
	e	16	14	3	0		15	22	1	3	
	f	5	1	0	0		4	4	0	0	
		165	168	9	15	7	172	167	14	8	7
20	a	65	65	0	5		63	59	8	1	
	b	42	31	1	1		27	47	5	2	
	c	34	46	2	1		38	40	1	1	
	d	54	31	6	1		48	29	3	1	
	e	4	3	0	1		9	3	0	1	
		199	176	9	9	5	185	178	17	6	6
21	a	19	38	1	2		37	39	3	0	
	b	31	37	2	2		37	51	4	2	
	c	52	48	4	1		36	38	2	2	
	d	65	53	3	2		60	53	4	5	
	e	19	13	0	1		22	7	1	0	
		186	189	10	8	5	192	188	14	9	6
22		159	134	5	6	4	185	135	9	6	4
23		142	115	4	2	2	135	106	4	5	4
24		16	13	0	0		10	9	0	0	
25		62	35	4	1	5	50	37	1	0	1
26		44	31	2	2	5	30	36	0	5	6
27		64	56	3	2	4	64	52	0	5	4
28		92	89	1	8	5	80	89	3	6	5
29		97	92	4	6	5	100	70	5	10	8
30		48	52	3	0	3	55	62	2	1	3
31		98	104	4	8	6	99	85	6	13	9
Total		1782	1625	73	91	5	1749	1571	107	92	6

On examining the numbers from the individual bottles only the normal fluctuation in the strength of linkage is noticeable.

Summary.

The strength of linkage between the factor for bifid wing and the factor for vermilion eye is approximately the same (viz. 32 units) in the original cross in its reciprocal, and in the F_2 from the crossovers of the original. (See Experiments I., II. and III., Tables II., III., V. and VI.)

The strength of linkage between yellow body color and bifid wing is constant (viz. 5 units) in the original and in the F_2 from the crossovers (See Experiment IV., Tables VII., VIII. and IX.).

A repetition of Experiment IV. showed a linkage value (viz. 6 units) not significantly different from that previously found. The linkage moreover is constant in the two reciprocal crosses of this experiment. (See Experiment V.; Tables X. and XI.)

The strength of linkage between white eyes and bifid wings is preserved in the crossovers (viz. original 8, crossover 9 units), but is different in the reciprocal (viz., 4). (See Experiments VI. and VII.; Tables XII., XIII. and XIV).

A modification of experiments VI. and VII. gave a strength of linkage (viz. 5 and 6), which approaches that of the reciprocal (viz., 4) but not the original linkage value (viz., 8). (See Experiments VIII. and IX.; Tables XVI. and XVIII.)

In all of the experiments of this paper the strength of linkage is apparently not changed by a previous crossing over between the factors in question.

The linkage value given by the females is the same as that given by the males of the same experiment. (See Tables XVI. and XVIII.)

Crossovers appear in the F_2 generation equally frequently among the first flies hatched as among those hatched last. That the factor for yellow body color has an effect on the viability of flies is evidenced from the deficient numbers of yellow flies in Tables VIII., X. and XI. A deficiency also occurs in flies with white eyes as compared with those possessing red eyes. (See Tables XIII., XVI. and XVIII.) A bifid wing factor, however, does not seem to have any such effect; the bifid winged flies comparing favorably with the long winged flies (see Table XVIII.).

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